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tctttctatt cataataaac tagatactgt tgatctttta aaaaaaaaaa aaaaaaaaaa 5821
aaaaaaaa (SEQ ID NO:6; GENBANK Accession No. NM 005544)

The double mutation of tyrosine 897 and 1180 was constructed by replacement of 3'-sequences coding 897F by the same region of 1180F using restriction enzymes NheI and EcoRI, and this construct was called 897F1180F orΔGrb2 ΔSyp. The expression plasmids were under control of a CMV promoter (hIRS-1-wt, ΔGrb2, ΔSyp, ΔGrb2, ΔSyp and pBK-CMV (mock) and linearized at the 3'-end of poly A signal sequences by MluI restriction enzymes followed by purification. A similar approach was used to change the tyrosine residue to